Copyright ©

Kieler Meeresforsch., Sonderh. 8, 46-54. Kiel 1991

Marine petroleum hydrocarbon degrading bacteria: distribution and activity in the North Sea and Baltic Sea

K. Bruns*, G. Dahlmann* and W. Gunkel**
*Bundesamt f. Seeschiffahrt und Hydrographie
Bernhard-Nocht-Str. 78, D-2000 Hamburg 4, Germany
**Biologische Anstalt Helgoland
Notkestr. 31, D-2000 Hamburg 52, Germany

Abstract

In 1988 and 1989 data about the distribution and activity of petroleum hydrocarbon degrading bacteria in the North Sea and Baltic Sea were collected. Crude oil degrading bacteria and the number of bacteria which especially degrade naphthalene were quantified using a modified dilution (MPN) method. Crude oil degrading bacteria were present in all of about 100 water samples, with as many as 10^3 ml^{-1} in sum. Naphthalene degrading bacteria were present in at least tenfold fewer numbers which corresponded with petroleum hydrocarbon (PHC) concentrations (ultraviolet fluorescence spectroscopy method, UVF) in more highly polluted areas. There is obviously a greater connection between this bacteria group and PHC pollution determined by UVF than between the more nonspecific group of crude oil degrading bacteria and UVF-determined PHC pollution. Data from the North Sea show an extremely high abundance of hydrocarbon degrading bacteria, even in winter, while in the southern Baltic Sea low numbers of bacteria were found and a slower crude oil degradation was observed.

Introduction

According to estimates by the Institute of Offshore Engineering (1984, loc. cit. SIDE et al. 1985) about 130 000 to 200 000 tons of petroleum hydrocarbons (PHC) enter the North Sea every year. For the Baltic Sea ENKELL (1987) names amounts between 20 000 to 66 000 tons per year (Tab. 1). For both seas the main sources are:
- land run-off, including industrial effluents, which accounts for 33.6 to 88.7 %,
- losses due to shipping accounting for 1.8 to 25.5 %,
- the atmospheric input which accounts for between 4.9 and 15.1 %.

Petroleum hydrocarbons in seawater are affected by several physical, chemical and biochemical processes. The extent of the resulting solubilization, adsorption, dispersion and degradation depends on the kind of hydrocarbons and the composition of the hydrocarbon mixture (summarizing publications: NATIONAL RESEARCH COUNCIL 1985, ATLAS 1984, SALOMONS et al. 1988, GIBSON 1984). Bacteria play an essential role in biochemical degradation processes. It is, how-

Table 1. Estimated input of petroleum hydrocarbons into the North Sea and Baltic Sea (x 10^3 t a^{-1}), modified according to [1] Institute of Offshore Engineering 1984, loc. cit. SIDE et al. 1985, [2] ENKELL 1987.

Source	North Sea[1]	Baltic Sea[2]
Natural seeps	0.3 - 0.8	-
Atmospheric	19	1 - 10
Rivers, land runoff (incl. inland municipal waste)	40 - 80	18 - 39
Coastal sewage discharges	3 - 14	
Coastal refineries	6	0.2
Oil terminal operations (incl. ballast water treatment)	0.8	0.1 - 0.2
Other coastal industrial effluent	9	0.7 - 1.3
Accidental losses from tankers at sea	5 - 12	0.2 - 9
Operational discharges from tankers at sea and losses from general shipping	21.8 - 33.8	0.16 - 6.5
Offshore production	23	<0.005
total:	127.9 - 198.4	20.3 - 66.2

ever, well known that temperature, oxygen-concentration and the availability of inorganic nitrogen and phosphorus limit their activity.

Our investigations focused on the distribution of PHC degrading bacteria and the PHC-concentration in their environment. We also studied the extent of alteration in the composition of crude oil which was incubated with marine bacteria. To this end, water samples were taken during research cruises in areas of the North Sea in winter 1988 and in spring 1989. Investigations in the Baltic Proper were carried out in spring 1989.

Material and methods

Petroleum hydrocarbons (PHC): Water sampling and the determination of the PHC-concentration were carried out as described in detail by STADLER and SCHOMAKER (1977) and DAHLMANN and LANGE (1981) using ultraviolet fluorescence spectroscopy (UVF). The PHC-concentration is expressed in Ekofisk crude oil equivalents as µg l^{-1} (IOC 1984).

Biodegraded crude oils were separated from the incubated water samples by solid phase extraction (Bond Elut C_{18}-cartridges, Analytichem, Harbor City, USA), elution with n-hexane/dichloromethane 1:1 (THEOBALD 1988), and analyzing by gas chromatography (GC) as described by BRUNS et al. (1989).

Bacteria: In water sampled with sterile bottles (1 l or 2 l, ZoBell sampler) or with sterile plastic bags (bacteriological Niskin sampler, General Oceanics, Miami, USA) in water depths > 100 m, three groups of bacteria were quantified: 1) the saprophytic heterotrophic bacteria as colony forming units (CFU) on 2216 E-M-Agar (GUNKEL 1964, RHEINHEIMER 1977). 2) the crude oil degrading bacteria as most probable number (MPN) with stripped Ekofisk crude oil and fuel oil 1:1 (GUNKEL and TREKEL 1967), and 3) the naphthalene degrading bacteria

with washed naphthalene as the sole carbon source. The liquid medium was modified by addition of 0.5 ml l^{-1} trace metal solution (COHEN-BAZIRE et al. 1957), which enhanced naphthalene decomposition activity (BRUNS 1986). Bacterial colonies were counted after three weeks and the MPN values were calculated after 6 weeks incubation at 18 °C.

Biodegradation experiments were performed in 1-l-bottles with 50 mg l^{-1} stripped Ekofisk crude oil and 500 ml pure water samples or samples which additionally contained inorganic nitrogen (1.31 µg l^{-1} N), phosphorus (3.05 µg l^{-1} P) and trace minerals (see above). The samples were slightly shaken or incubated in a vertical rotating apparatus at 18 °C.

Results and discussion

PHC-concentration and PHC degrading bacteria

In winter 1988 we investigated the distribution of PHC degrading bacteria (Fig. 1, bars in logarithmic scale) and the PHC-concentrations (listed beyond the bars as µg l^{-1}) at a depth of 1 m in areas of the North Sea and Irish Sea.

In every sample we found CFU in a range of 2 - 20 000 N ml^{-1} as well as crude oil degrading bacteria in a range of 0.03 - 430 N ml^{-1}. Naphthalene degrading bacteria were detected only in 30 out of 45 samples with a maximum of 230 bacteria per ml.

In general we found high numbers of bacteria in higher polluted areas (German Bight, estuaries of Rhine and Thames, Irish Sea). Detection of the naphthalene degraders (filled bars) was mainly possible when the PHC-concentration was higher than 1 µg l^{-1} in contrast to the crude oil degrading bacteria which were even found in water samples containing less than 0.4 µg l^{-1} PHC.

The amounts of PHC in the surface water (5 m) of the Baltic Proper in spring 1989 were low (Fig. 2, bottom of bars) compared to those found in the North Sea. The concentrations revealed a uniform distribution of PHC between 1.07 - 2.95 µg l^{-1} which can be attributed to the relatively high atmospheric input into the Baltic Sea. The numbers of bacteria we found were lower than in the North Sea but they still reflected the influence of the coast: higher bacteria numbers in coastal zones, at the entrance to the Gulf of Finland and the Gulf of Bothnia and lower numbers in the central Baltic Proper (Fig. 2). In accordance with the low PHC-concentrations in the surface samples, we found bacterial communities which were dominated by the heterotrophic group (7 - 490 CFU ml^{-1}). Again crude oil degrading bacteria were detected in all samples in the 0.091 - 23 N ml^{-1} range in contrast to the naphthalene degrading bacteria which were present only in 20 out of 24 samples in the 0.023 - 23 N ml^{-1} range.

Parallel estimates of PHC-concentrations and PHC degrading bacteria reflect both the ecological stress and the biological reaction of the system. Nevertheless, it is difficult to find a correlation between the number of bacteria and the PHC-concentration measured in the water sample. Only those bacteria which especially degrade naphthalene were barely present in samples with low PHC-concentrations less than 1 µg l^{-1}, whereas the group of crude oil degrading bacteria was present every time. We conclude from this that naphthalene degrading bacteria might be suitable organisms for indicating high PHC-polluted areas.

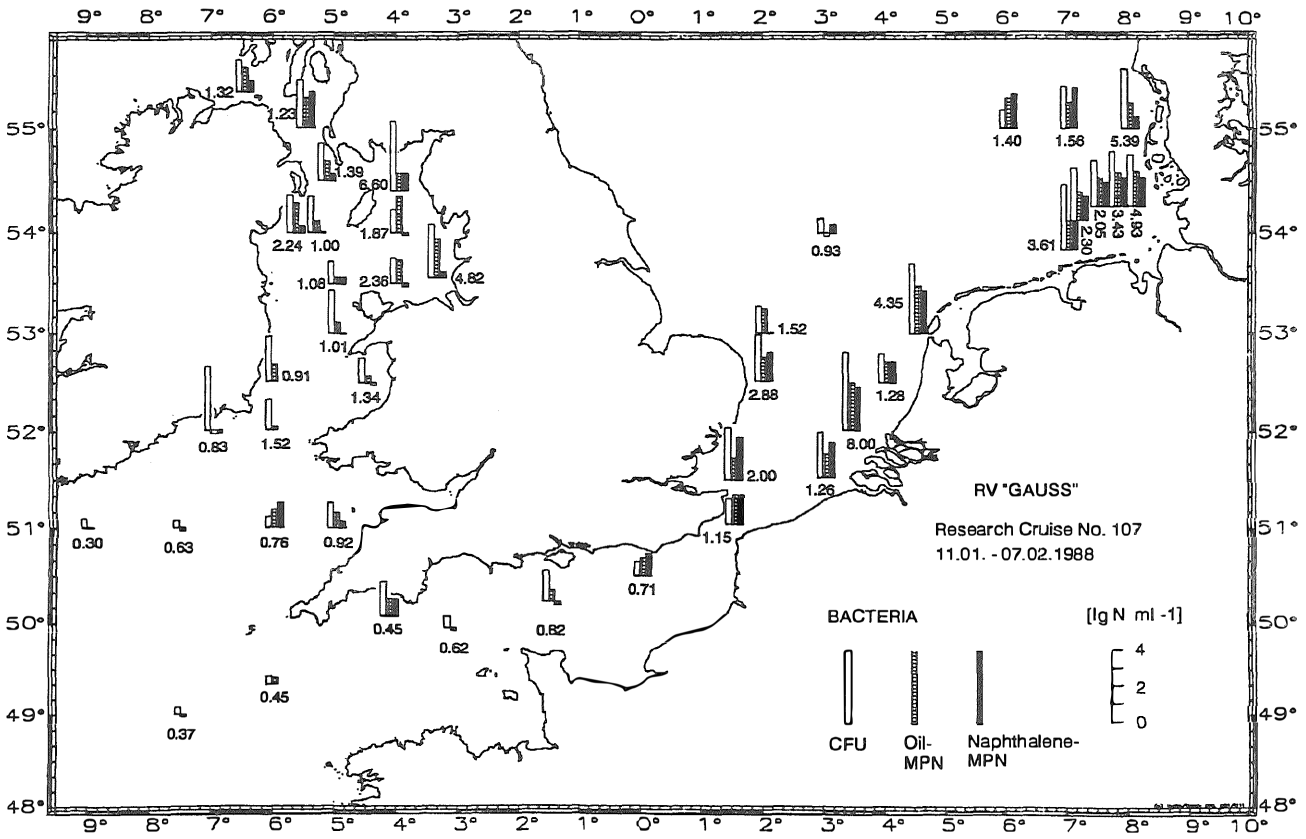

Fig. 1. Section of stations sampled at a depth of 1 m during a cruise with RV "GAUSS" in January 1988 in the North and Irish Seas: distribution of heterotrophic (CFU), crude oil (oil-MPN) and naphthalene degrading bacteria (naphthalene-MPN) as lg N ml^{-1}. Numeric PHC-concentrations ($\mu g\ l^{-1}$) below the plotted bars.

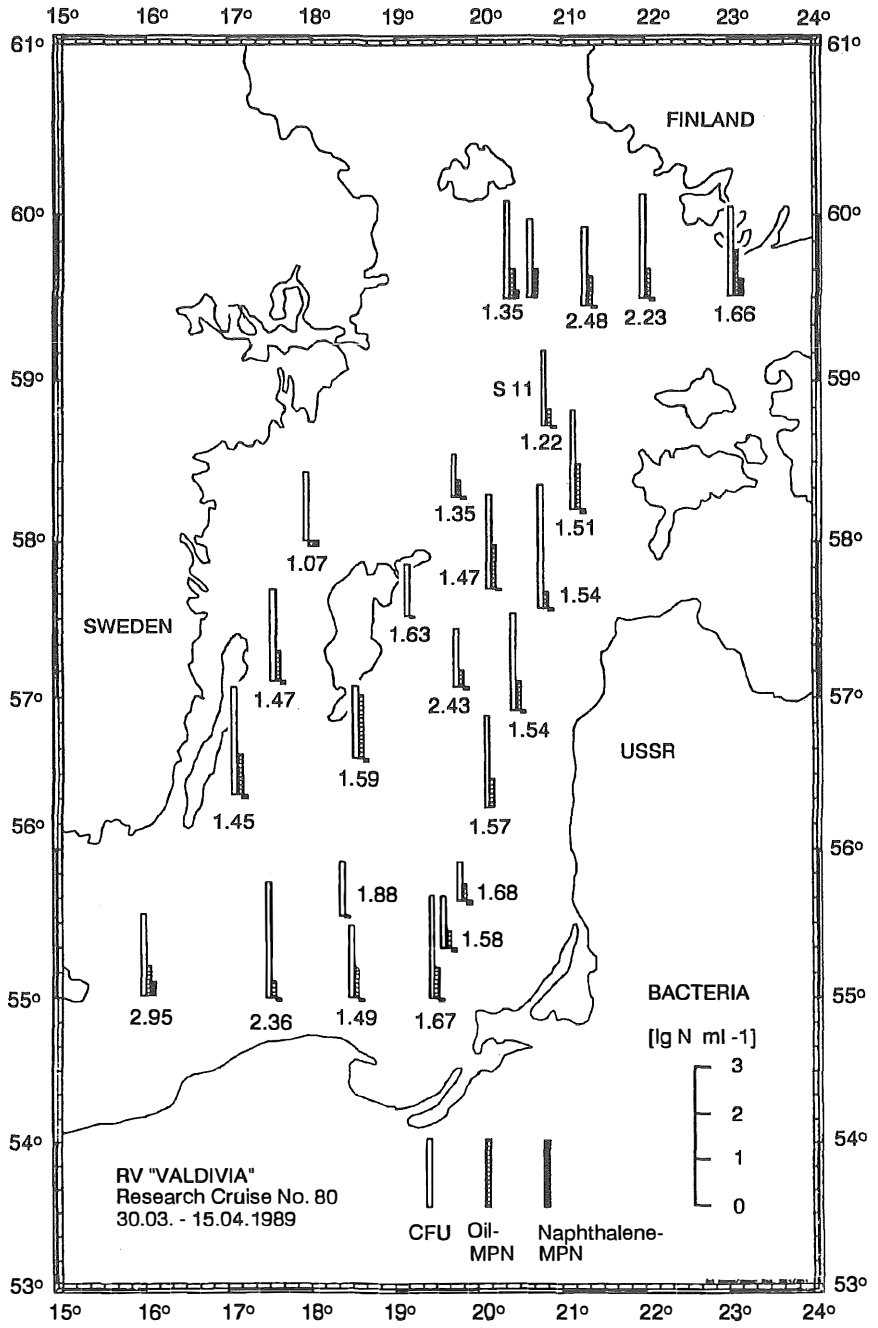

Fig. 2. Stations sampled at a depth of 5 m during a cruise with RV "VALDIVIA" in April 1989 in the Baltic Proper (further explanations see Fig. 1).

Degradative activity

As reported above, PHC degrading bacteria could be detected in every water sample. Does this mean, however, that every bacterial community, with the exception of the limitations mentioned above, can decompose crude oil to the same extent?

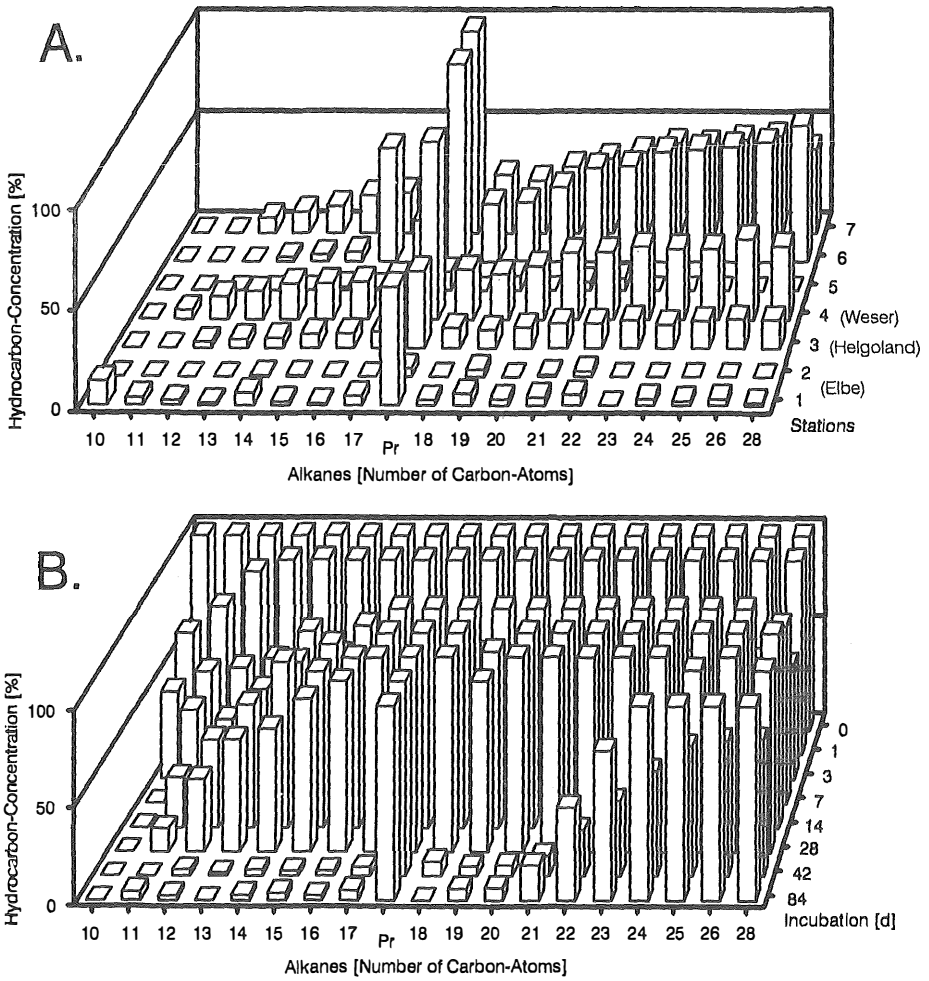

Fig. 3. Degradation of Ekofisk crude oil by bacteria in spring 1989 in samples from the German Bight (A) and from the Baltic Proper (B) with additionally available inorganic nitrogen and phosphorus: concentration of crude oil alkanes (as % of the initial value, y-axis) with different carbon-atom-numbers or pristane (PR) (x-axis) after biodegradation in samples from several stations after 14 days incubation (A) or in samples after several periods of incubation (B) (z-axis).

After 14 days incubation of Ekofisk crude oil with samples taken from several locations on a transect between the Elbe and Weser estuaries and the open sea in spring 1989 the expected correlation between the gradient of inorganic nutrients and biodegradation was obvious: the n-alkanes were reduced by 70 to 90 % in samples from areas affected by the estuaries; near Helgoland, only 30 to 50 % of the n-alkanes were decomposed. In water samples from the open sea only the alkanes with less than 14 C-atoms were slightly decomposed. This corresponds with the results of a long term incubation of samples we also took from a station in the Baltic Proper in spring 1989 (St. 11 in the latitude of the Gulf of Riga, see Fig. 2). But with additionally available nitrogen and phosphorus in the samples from the German Bight and the Baltic Proper, the degradative activity revealed remarkable differences (Fig. 3). In samples from the open sea of the German Bight (Fig. 3A, St. 5-7), the n-alkanes were reduced by 50 % at least after only 14 days incubation. Moreover, the branched chain alkane pristane (PR), known to be more resistant to biodegradation, was reduced. Compared with this, the alkane concentrations in the Baltic Sea samples remained almost the same as before after the twofold incubation period (Fig. 3 B). This was unexpected because the bacteria from all groups increased 10 000-fold within 3 days and remained stable over a period of approximately 4-6 weeks as was observed on repeated occasions for bacteria sampled near Helgoland (BRUNS et al. 1989, GUNKEL and DAHLMANN 1987). They decomposed the alkanes at least to the same extent as in the open sea of the German Bight (Fig. 3 A, St. 3, 5-7).

The low extent of crude oil degradation by Baltic Sea bacteria even when nitrogen and phosphorus were added (Fig. 3 B), was determined for one location only; the results cannot be applied to the whole Baltic Sea. Although the results correspond with those of TSYBAN and IZRAEL as cited in GOCKE et al. (1990), further investigations in areas with direct coastal influences are necessary to compare the degradative efficiency of bacterial communities in the North Sea and Baltic Sea.

In the German Bight it was obvious that an intensive microbial PHC degradation occurs only very near the coast owing to the amounts of inorganic nitrogen and phosphorus available there. Provided that sufficient amounts of inorganic nitrogen and phosphorus are available, bacteria in the open sea are also able to decompose PHC. It is remarkable that in this case the degradative efficiency of bacteria in the open sea did not match that of the bacteria sampled near the coast (Fig. 3 A). With regard to the results of the Baltic Proper, this suggests that besides the known limitations of microbial PHC degradation, other factors as yet unrecognized influence the biodegradation of PHC in the sea. The preadaptation of microorganisms in permanently polluted areas (HUDAK and FUHRMAN 1988) and the selective mechanisms of the bacterial loop (FENCHEL 1982) should be considered as additional factors.

In general, the proportion of microorganisms involved in the decomposition of PHC in water of the open sea seems to be very low. This is why photooxidation may indeed be more important in the degradation of PHCs in the open sea than microbial degradation processes as discussed by TJESSEM and PALMORK (1984).

References

ATLAS, R.M., 1984. Petroleum Microbiology. Macmillian Publishing Company, New York.

BRUNS, K., 1986. Einflüsse von Naphthalin auf marine Bakterien. Master Thesis, Justus-Liebig-Universität Gießen, FRG.

BRUNS, K., W. GUNKEL, G. DAHLMANN and N. THEOBALD, 1989. Biologischer Abbau von Erdölkohlenwasserstoffen in der Nordsee unter besonderer Berücksichtigung von schwer abbaubaren Komponenten, die als Leitsubstanzen für die chemisch analytische Überwachung der Meeresumwelt dienen sollen. Wissenschaftlich-Technische Berichte, 1989-3. Deutsches Hydrographisches Institut, Hamburg, FRG.

COHEN-BAZIRE, G., W.R. SISTROM and R.Y. STANIER, 1957. Kinetics studies of pigment synthesis by non-sulphur purple bacteria. J. Cell. Comp. Physiol. 49, 25-68.

DAHLMANN, G. and W. LANGE, 1981. Investigation of the distribution of petroleum hydrocarbons in the German Bight by means of fluorescence spectroscopy. Dt. hydrogr. Z. 34, 150-161.

ENCKELL, E., 1987. Oil pollution load on the Baltic Sea; a compilation of measured and estimated load. Baltic Sea Environ. Proc. 22, 2-9.

FENCHEL, T., 1982. Ecology of heterotrophic microflagellates IV. Quantitative occurence and importance as bacterial consumers. Mar. Ecol. Prog. Ser. 9, 35-42.

GIBSON, D.T., 1984. Microbial degradation of organic compounds. Marcel Dekker Inc., New York.

GOCKE, K., A. HEINÄNEN, K.-O. KIRSTEIN, M. MACIEJOWSKA, G. PANOV and A. TSYBAN, 1990. Second periodic assessment of the state of the marine environment of the Baltic Sea, 1984 - 1988; General conclusions, 7. Microorganisms. In: Baltic Marine Environment Protection Commission - Helsinki Commission - (ed.), Baltic Sea Environment Proceedings No. 35 A.

GUNKEL, W., 1964. Einwirkungen des kalten Winters 1962/63 auf die Bakterienpopulationen vor Helgoland. Helgoländer wiss. Meeresunters. 10, 246-256.

GUNKEL, W. and G. DAHLMANN, 1987. Bacterial degradation of heavy fuel oil in seawater. Baltic Sea Environ. Proc. 22, 68-80.

GUNKEL, W. and H.H. TREKEL, 1967. Zur Methodik der quantitativen Erfassung ölabbauender Bakterien in verölten Sedimenten und Böden, Öl-Wassergemischen, Ölen und teerartigen Substanzen. Helgoländer wiss. Meeresunters. 16, 336-348.

HUDAK, J.P. and J.A. FUHRMAN, 1988. Effects of four organic pollutants on the growth of natural marine bacterioplankton populations. Mar. Ecol. Prog. Ser. 47, 185-194.

Intergovernmental Oceanographic Commission, 1984. Manual for monitoring oil and dissolved/dispersed petroleum hydrocarbons in marine waters and on beaches. - Manuals and Guides No. 13. IOC/UNEP, Paris.

National Research Council, 1985. Oil in the sea, inputs, fates, and effects. National Academy Press, Washington D.C.

RHEINHEIMER, G., 1977. Microbial ecology of a brackish water environment. Springer Verlag, Berlin.

SALOMONS, W., B.L. BAYNE, E.K. DUURSMA and U. FÖRSTER, 1988. Pollution of the North Sea - An assessment. Springer-Verlag, Berlin.

SIDE, J., Ch. HERD and W. GROGAN, 1985. Oil spill pollution: The North Sea experience of cooperative measures, 615-620. In: American Petroleum Institute (ed.), Proc. 1985 Oil spill conference. American Petroleum Institute, Washington D.C.

STADLER, D. and K. SCHOMAKER, 1977. Ein Glaskugelschöpfer zur kontaminationsfreien Entnahme von Seewasser unter der Oberfläche für die Analyse von Kohlenwasserstoffen und halogenierten Kohlenwasserstoffen. Dt. Hydrogr. Z. 30, 20-25.

THEOBALD, N., 1988. Rapid preliminary separation of petroleum hydrocarbons by solid-phase extraction cartridges. Anal. Chim. Acta 204, 135-144.

TJESSEM, K. and K.H. PALMORK, 1984. An overview of auto/photooxidation of petroleum in the marine environment. ICES, Marine Chemistry Working Group, 1-25.